Seedling Nursery Culture of Whitebark Pine at Dorena Genetic Resource Center: Headaches, Successes, and Growing Pains

Riley, Lee E.[1]; Coumas, Carmen M.[1]; Danielson, Judith F.[1]; and Berdeen, John C.[1]

[1]USDA Forest Service, Dorena Genetics Resource Center, Cottage Grove, OR 97424

Abstract

The production of whitebark pine (*Pinus albicaulis*) seedlings for white pine blister rust (*Cronartium ribicola*) resistance screening and reforestation has required the development of nursery culturing regimes to optimize both germination and subsequent growth. Germination and culturing studies have been conducted to modify and adapt growing regimes to the environment of USDA Forest Service Dorena Genetic Resource Center. As a result, seedlings of sufficient height and caliper are now produced in 2 years for both blister rust resistance screening and outplanting on USDA Forest Service and USDI National Park Service lands.

Introduction

Production of whitebark pine (*Pinus albicaulis*) seedlings in nurseries, for both disease resistance screening and reforestation, requires overcoming some inherent problems of regeneration in the species. Seed germination is generally both poor and erratic; only 10% to 15% germinate in the first year under natural conditions (McCaughey 1993, cited in Burr and others 2001). The presumed reasons for this include: predation (and caching) of seed crops before embryo maturity by various animals, lack of appropriate substrate and climatic conditions, complex dormancy-release physiological requirements, and extremely hard seedcoats. These factors can be positively adaptive in a natural environment over a period of time by providing a small supply of germinants over a period of 2 to 3 years, given the proper conditions. However, they present serious challenges in a nursery environment.

Depending on various factors, up to 100% germination can be achieved under strict laboratory conditions followed by intensive greenhouse culturing and monitoring (McCaughey 1992; Riley unpublished data). The challenge has been to determine a regime that is reliably successful, in an offsite environment, for a broad range of seedlots. Previous literature on attempts to successfully produce whitebark pine seedlings is not extensive, and determining a routinely satisfactory protocol has been difficult. Stratification regimes and seed pretreatments vary (Krugman and Jenkinson 1974; Arno and Hoff 1989; Rose and others 1998), and little research has been done on culturing regimes. The most successful procedures have been those developed by USDA Forest Service Coeur d'Alene Nursery in Coeur d'Alene, Idaho (Burr and others 2001). However, other nurseries must adapt growing regimes to fit their particular climates. USDA Forest Service Dorena Genetic Resource

Center (DGRC), located in western Oregon at 43°47', -122°58', elevation 285 m, spent several years adapting these regimes to the growing environment at that location.

Initial Attempts at Seedling Culture

In cooperation with the USDA Forest Service and USDI National Park Service, Dorena Genetic Resource Center has sown and cultured approximately 178 families of whitebark pine, from a wide range of locations throughout the Pacific Northwest from 1996 to 2006. Seedlings have been used in white pine blister rust (*Cronartium ribicola*) resistance screening as well as outplanting on Federal lands. Due to limited information resources and a number of environmental variables, early sowings resulted in low germination and few viable seedlings.

Direct Sowing into Rust Testing Frames

Although DGRC has been expanding the rust resistance testing program over the years to include other five-needle pines, western white pine (*Pinus monticola*) and sugar pine (*P. lambertiana*) have been the traditional species sown for standard testing. Nursery protocols for these species have been modified over the years. Following appropriate seed handling and stratification, standard nursery practice includes hand-sowing of both species directly into 1 x 1.2 x 0.3 m testing frames located in an outside growing area and culturing for two years prior to inoculation with rust spores (Figure 1).

In 1996, 2 seedlots of whitebark pine were available for blister rust testing at DGRC. Two hundred seeds from these lots were soaked in 1% H_2O_2 for 24 hours, rinsed, and soaked in water for an additional 24 hours. They were then placed in cold stratification at 1 °C for 90 days, hand-sown directly into rust testing frames with western white pine, and cultured under standard nursery regimes for western white pine and sugar pine. Approximately 5% of the seeds from all 3 lots germinated, resulting in insufficient seedlings for rust testing (Figure 2).

Fig. 1. Hand-sowing of western white pine seeds in frames for blister rust testing.

Fig. 2. Direct-sowing of whitebark pine seeds into rust testing frames with western white pine. Whitebark pine is located in the 3rd row from the left.

Direct Sowing into Seedling Containers

In 2000, the decision was made to sow whitebark pine seeds into containers to culture under greenhouse conditions for rust testing. Two hundred seeds from each of 6 lots of whitebark pine were soaked in 1% H_2O_2 for 24 hours, rinsed, and soaked in water for an additional 24 hours. They were then placed in cold stratification at 1 °C for 90 days, scarified using a razor blade to remove a portion of the seedcoat, and sown directly into Ray Leach SuperCells® (164 cm^3) (Stuewe and Sons, Inc., Corvallis, OR) in a greenhouse. As with the 2000 sowing, the resulting germination was extremely low (Figure 3A). Approximately 11% of the seeds in these seedlots did germinate and emerge, but several were damaged by both mice and birds in the greenhouse (Figures 3B and 3C).

A B C

Fig. 3. Extremely low germination resulting from hand sowing whitebark pine seeds directly into containers following stratification and scarification (A). The few germinants that did emerge were damaged by both birds (B) and mice (C).

Culturing Trials

2002 Whitebark Pine Cultural Regimes Study

Due to the failures of the 2 previous attempts to germinate and culture whitebark pine at DGRC, a study was implemented in 2002 to determine seed pre-treatment, stratification, germination, and culturing regimes that would provide optimal conditions for germination and growth. This study was based on the protocols developed by Burr and others (2001) and Burr (2002) at USDA Forest Service Coeur d'Alene Nursery (CDAN). The objective was to compare CDAN protocols with other pre-sowing and post-sowing treatments to determine proper growing procedures in a different growing environment and with seedlots from different provenances.

Materials and Methods

Twenty seedlots of whitebark pine were used in this study. The seedlots ranged in storage time from 0 to 7 years. Nineteen of the lots were individual tree collections from 5 forests in Washington and Oregon; one lot was a bulk seedlot received from CDAN.

Seeds from each lot were X-rayed on a template to extract only filled seeds. A total of 504 filled seeds from each whitebark pine seedlot were divided into a total of 24 treatments.

Seed pretreatment. Seeds from all lots were divided equally into 2 treatments (252 seeds each). In treatment 1 (control as utilized by CDAN), seeds from each lot were placed in labeled mesh bags and soaked for 48 hours in running tap water to remain aerated during water absorption. In treatment 2 (standard procedure at DGRC), seeds from each lot were placed in labeled mesh bags, soaked for 24 hours in a 1% H_2O_2 solution, rinsed, and then soaked for an additional 24 hours in water. All seeds were rinsed once per week with water during stratification, and moldy seeds were rinsed well with 1% H_2O_2

Stratification time. All seeds were placed in warm stratification (20 ^oC night/22 ^oC day) for 28 days (Burr and others 2001). Following the warm stratification period, seeds were further subdivided (from different soaking treatments) into 3 cold stratification periods: 30 days, 60 days (control), and 90 days at 1-2 ^oC (84 seeds each).

Germination temperature. At the conclusion of all stratification periods, seeds were nicked with a scalpel or razor blade, approximately 1 mm from the radicle end, along the main line dividing the 2 halves of the seedcoat and placed on moistened blotter paper in 10 x 10 cm clear plastic boxes. Seeds were further subdivided into 2 treatments (42 seeds each). Boxes containing the control seeds (CDAN) were placed in a germinator maintained at 20 ^oC night/22 ^oC day with a 12-hour photoperiod. Boxes containing seeds in treatment 2 were placed in a germinator maintained at 16 ^oC night/18 ^oC day with a 12-hour photoperiod.

All lots were monitored for germination every 48 hours. Seeds were considered germinated when the radicle protruded from the seedcoat to a length of 2 mm. Germinated seeds were removed from the germination boxes, planted into Ray Leach SuperCells® (164 cm^3) containing pre-fertilized and pre-moistened "seedling mix," and covered with 0.3 to 0.6 cm nursery grit. Following planting, supercells were maintained at a constant temperature of 21 ^oC for 7 days with 18 hour photoperiod in a building, and then moved to greenhouses.

Culturing. Two different greenhouse environments were tested during this study. Cells containing germinated seeds were further subdivided (up to 21 seeds each) into either a climate-controlled greenhouse (with daytime temperatures ranging from 21 to 27 °C throughout the growing season) or a standard greenhouse subject to ambient conditions (with daytime temperatures ranging from 21 to 38 °C throughout the growing season).

Results

Overall germination percentage was significantly different ($P<0.0001$) between seedlots. Although germination percentage based on age of the seedlots was confounded by geographic origin, some older seedlots ranged from 80% to 100% germination in some treatments.

Seed pretreatment. No significant difference was found in total germination percentage between seed pretreatments. However, the standard DGRC pretreatment of 24 hour soak in H_2O_2 resulted in slightly higher overall germination and slightly faster speed of germination.

Stratification time. There were significant differences ($P<0.0001$) between stratification treatments in overall germination percentages and speed of germination. The 120-day

stratification treatment yielded both higher germination percentages and speed of germination than either the 90-day or 60-day stratification periods. Total germination for seeds stratified for 120 days ranged from 44.6% to 97.0%, with an overall average of 68.9%; seeds stratified for 90 days ranged in germination from 31.5% to 81.5%, with an average of 61.6%; seeds stratified for 60 days ranged in germination from 21.4% to 54.2%, with an average of 36.9%. In addition, depending on the seedlot, the 120-day stratification period resulted in higher numbers of seeds that did not require scarification due to seedcoat splitting.

Germination temperature. No significant difference was found in either total germination percentage or speed of germination between germination temperature regimes. However, the higher night/day temperatures (20 ^{o}C/22 ^{o}C) resulted in higher numbers of moldy seeds during the monitoring period.

Culturing. Seedlings from all seedlots and germination treatments that were placed in the climate-controlled greenhouse were significantly shorter in height at the end of the growing season than those grown in ambient temperatures. Average heights (by seedlot) for seedlings grown in the climate-controlled house ranged from 2 to 4.5 cm, with an overall average of 3.7 cm; average heights (by seedlot) for seedlings grown at ambient temperatures ranged from 5.2 to 7.1 cm, with an overall average of 6.3 cm. However, observationally, it appeared that budset occurred earlier in seedlings subjected to the higher greenhouse temperatures.

The results of the culturing portion are somewhat counter-intuitive. One explanation for the decreased growth rates in the climate-controlled greenhouse may be that the greenhouse was used for other purposes during that growing season. As a temporary measure, the seedlings were grown on pallets on the floor of the greenhouse directly in front of the cool cells. The resulting cooler temperatures in this location may have precluded the proper release of the control-release fertilizer in the cells.

2006 Whitebark Pine Scarification Study

Depending on the seedlot, many seeds require scarification to increase germination potential. The majority of seedlots sown for testing at DGRC are small lots; each lot is usually comprised of less than 200 seeds. Since scarification methods used for larger bulk lots are impractical, each seed must be hand-nicked if the seedcoat has not cracked during stratification.

Nicking seeds with a scalpel presents concerns for both human safety as well as the possibility of damaging the megagametophyte tissue prior to germination. Nicking is also very slow and labor intensive. For these reasons, a small trial was implemented to determine if light abrasion of the seedcoat caused by sanding would produce similar or better germination potential as nicking seeds with a scalpel.

Materials and Methods

In spring 2006, 8 individual seedlots from Crater Lake National Park and 1 bulk lot from the Deschutes National Forest were used in a scarification study. Fifty seeds from each of the 9 lots were stratified for 30 days at 10 °C and 90 days at 1-2 °C. Following stratification, 25 seeds from each lot were nicked with a scalpel using the standard protocol, and 25 seeds

were lightly hand-sanded using 100-grit sandpaper. Seeds were then placed on moistened blotter paper in labeled 10 x 10 cm clear plastic boxes. Boxes were placed in a germinator maintained at 16 °C night/18 °C day with a 12-hour photoperiod. Germination was monitored twice per week for 4 weeks.

Results

Germination differed significantly between seedlots, with germination percentages ranging from 14% to 84%. However, no significant difference was found between scarification treatments. The seed abrasion treatment appeared to yield similar results to the hand-nicking, but was much faster and easier.

Current Cultural Methods

Culturing regimes for whitebark pine at DGRC have evolved since 2000 based on experience, as well as the results of culturing studies and trials. These regimes may not be applicable to other situations, but appear to work well in the growing climate at DGRC. Current methods used at DGRC are detailed below. For further information on terminology and physiological details of whitebark pine seedling culture, refer to Burr and others (2001).

Stratification

Stratification is defined as a seed pregermination treatment to break seed dormancy and provide for uniform germination, and is accomplished by placing seeds in a moist cool environment (often preceded by a warm period) to hasten afterripening (Schopmeyer 1974; Bonner 1984). Seeds at DGRC are placed in individually labeled mesh bags (Figure 4), soaked for 24 hours in 1% H_2O_2, rinsed, and soaked an additional 24 hours in water to take up water as well as reduce the levels of seedborne pathogenic fungi. Mesh bags are hung on dowels in covered plastic tubs, placed in warm stratification at 10 °C for 30 days, then at 1 to 2 °C for 90 days. The warm stratification temperature is actually cooler than temperatures tested in previous cultural trials. This temperature appears to reduce the occurrence of mold as well as the number of early germinants. All seeds are rinsed once per week with water during stratification, and moldy seeds are rinsed well with 1% H_2O_2.

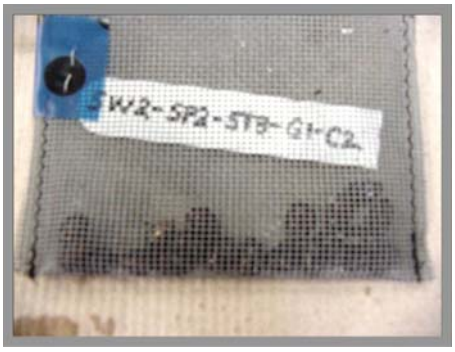

Fig. 4. Individually labeled mesh bag for seed stratification.

Scarification and Germination

When stratification is complete, the seedcoat of each seed is abraded, using 100-grit sandpaper, at the radicle end (approximately 1 mm back from the tip) and along the main line dividing the 2 halves of the seedcoat (Figure 5A). Care must be taken to avoid sanding too heavily, as damage to the megagametophyte tissue could result. This process is done to help maximize germination percentage as well as uniformity of germination. Seedlots which are not sanded or nicked show lower germination percentage and germinate at a slower rate (Burr and others 2001). A few seedlots have a percentage of seeds that begin to crack during

stratification. These seeds are not sanded. During the 2007 sowing season, a newly designed sanding machine will be used for seedcoat abrasion (Figure 5B) (Spence 2006).

Sanded seeds are placed on moistened blotter paper in 10 x 10 cm clear plastic boxes (Figure 6A), which are then placed in a germinator maintained at 16 °C night/18 °C day with a 12-hour photoperiod (Figure 6B).

A B

Fig. 5. Seedcoat abrasion using 100-grit sandpaper (A). Sanding machine, designed and built at DGRC, to be used for seedcoat abrasion (Spence 2006) (B).

A B

Fig. 6. Whitebark pine seeds in individually labeled germination boxes (A), which are thenplaced in a germinator maintained at 16 °C night/18 °C day with a 12-hour photoperiod (B).

Seedlots are monitored for germination twice per week for 5 weeks, and are considered germinated when the radicle protrudes from the seedcoat to a length of 2 mm and is curved (Figure 7). Germinated seeds are removed from the germination boxes, sown into individually labeled Ray Leach SuperCells® (164 cm^3) containing pre-fertilized (180-day Nutricote® control-release fertilizer [18-6-8 with minors]) and pre-moistened media (peat:composted fir bark:perlite:pumice [40:20:20:20]), and covered with nursery grit (Figure

8). Following sowing, supercells are placed in racks on covered tables inside a heated building and kept at approximately 21 °C (18-hour photoperiod) for 7 days.

At the end of this period, racks are moved to a greenhouse with lights (18-hour photoperiod) for the remainder of the growing season. All tables are covered with a mesh screen prior to rack placement, and racks are covered with mesh screen tops to protect seeds from birds and rodents (Figure 9). Covers remain in place until seedcoats have been shed from the seedlings (approximately 2 weeks following emergence).

Fig. 7. Germinated whitebark pine seeds prior to sowing.

Fig. 8. Sowing germinated seed.

Fig. 9. Seedling protection in the greenhouse.

Culturing—Year One

During the first growing season, supplemental light is used in the greenhouse (18-hour photoperiod) from germination through the middle of October. Supplemental lighting is used to mimic high elevation growing conditions as well as extend the growing season outside the normal range of the species.

Seedling nutrition includes the initial application of control-release fertilizer prior to sowing, as well as a variety of soluble fertilizers, depending on the growth stage, throughout the growing season. Fertilization is initiated when seedcoats have been shed from the seedlings. Low vigor seedlings may have difficulty shedding seedcoats (Burr and others 2001), so it may be necessary to remove seedcoats by hand. Soluble fertilizers are applied once per week through the middle of October.

Seedlings are overwintered in a greenhouse with the sides open to achieve appropriate chilling hours.

Culturing—Year Two

Fig. 10. Fertilization of individual cells at the beginning of the second growing season.

During mid-February of the second growing season, the greenhouse sides are closed, although no supplemental heat is added to the growing environment. Seedlings begin to receive supplemental lighting (18-hour photoperiod), which will continue through the middle of August. Nutricote® control-release fertilizer 140-day (18-6-8 with minors) is added to each cell by hand (Figure 10).

Seedling nutrition includes the application of control-release fertilizer, as well as a variety of soluble fertilizers, applied once per week through the middle of August. During the middle of August, seedlings are moved to an outdoor growing situation for hardening-off prior to either disease resistance testing or outplanting.

Seedling Results

The cultural regimes currently in use at DGRC have resulted in 2-year-old seedlings of sufficient height and caliper for use in either rust testing or outplanting (Figure 11). Two-year seedling heights range from a minimum of 6 cm to a maximum of slightly over 14 cm depending on seed source (Figure 12). To date, 437 whitebark pine seedlots (401 individual tree selections) have been sown and cultured for rust testing and/or outplanting on USDA Forest Service and USDI National Park Service lands. In 2007, an additional 247 seedlots are scheduled to be sown for testing and outplanting in 2008.

Fig. 11. Two-year old seedlings grown at Dorena Genetics Resource Center.

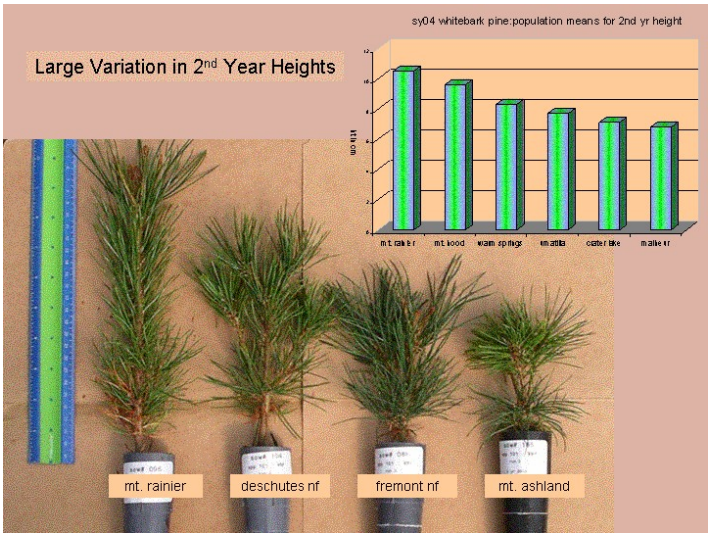

Figure 12. Variation in 2nd year height growth, depending on seed source.

References

Arno SF, Hoff RJ. 1989. Silvics of whitebark pine (*Pinus albicaulis*). Ogden (UT): USDA Forest Service Intermountain Research Station. General Technical Report INT-253. 11 p.

Bonner FT. 1984. Glossary of seed germination terms for tree seed workers. New Orleans (LA): USDA Forest Service, Southern Forest Experiment Station. General Technical Report SO-49. 4 p.

Burr KE. 2005. Personal communication. Coeur d'Alene (ID): Horticulturist, USDA Forest Service, Coeur d'Alene Nursery.

Burr, KE, Eramian A, Eggleston K. 2001. Growing whitebark pine seedlings for restoration. In: Tomback DF, Arno SF, Keane RE, editors. Whitebark pine communities. Washington (DC): Island Press. p 325-345.

Krugman SL, Jenkinson JL. 1974. Pinus L. Pine. In: Schopmeyer CS, technical coordinator. Seeds of woody plants in the United States. Washington (DC): USDA Forest Service. Agriculture Handbook No. 450. p 598-638.

McCaughey WW. 1992. The regeneration process of whitebark pine. In: Schmidt WC, Holtmeier F-K, compilers. Proceedings—international workshop on subalpine stone pines and their environment: the status of our knowledge; 1992 Sept 5-11; St Moritz, Switzerland. Ogden (UT): USDA Forest Service, Intermountain Research Station. General Technical Report INT-GRT-309. p 179-187.

McCaughey WW. 1993. Whitebark pine regeneration—results from a study on the Gallatin National Forest. Nutcracker Notes 1:5-6.

Rose R, Chachulski CEC, Haase DL. 1998. Propagation of Pacific Northwest native plants. Corvallis (OR): Oregon State University Press. 248 p.

Schopmeyer CS (technical coordinator). 1974. Seeds of woody plants in the United States. Washington (DC): USDA Forest Service. Agriculture Handbook No. 450. 883 p.

Spence R. 2006. Personal communication. Cottage Grove (OR): Maintenance Supervisor, Dorena Genetic Resource Center.